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The Acute Toxicity of Allyl Chloride by Subcutaneous Injection in Mice

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Abstract Male ICR mice were administered allyl chloride at dose of 496 mg/kg, 600 mg/kg, 720 mg/kg, 864 mg/kg or 1037 mg/kg by a single subcutaneous injection.

Sixteen of 25 mice died by the 7th day after the injection and LD_{50} was calculated 621 mg/kg body weight (95% C. I. : 522-739 mg/kg). A marked congestion with severe hemorrhage and edema were observed in the lung. Liver and kidney damages were also found, and these were characterized by the dilated sinusoids, degenerative change of hepatic cells, and focal necrosis in the liver ; and necrosis of epithelium in convoluted tubules of the kidneys.

Nine mice have survived by the 7th day after the injection and all of them showed a various degree of damages in the testes. The testicular lesions could be classified into two types. One type of the lesion was characterized by degeneration and exfoliation of germ cells, appearance of polynuclear giant cells in the seminiferous tubules, and mild proliferation of Leydig cells in the interstitium. In another type of the lesion, all type of cells in tubules, including Sertoli cells, and Leydig cells became necrotic. This is the first study reporting the testicular toxicity of allyl chloride.

Introduction

Allyl chloride is a highly volatile unsaturated aliphatic hydrocarbon compound. This compound has been used extensively as a chemical intermediate for epichlorohydrin (the major use) and other chemicals (e. g. pesticides and pharmaceuticals). He et al.[6),8)] reported polyneuropathy in men after chronic occupational exposure to allyl chloride vapor. Liver[5)] and kidney[3)] damages were also reported by the occupational exposure to this compound.

In animal experiments, like human cases, peripheral neuropathy[6),7),9),11)] were reported along with liver[12)] and kidney[7),12)] damages in repeated inhalation exposure to allyl chloride. Adams et al.[1)] also reported severe damage of the lung, severe degenerative change of the kidney and mild degenerative change of the liver as the acute vapor toxicity of this compound, though there are few reports about the acute toxicity of allyl chloride.

Therefore, we evaluated the acute toxicity of allyl chloride in mice by subcutaneous injection in this study.

Materials and Methods

Animals

Male ICR mice (Crj : cd-1), five weeks of age, were purchased from Charles River Japan, Inc. (Yokohama, Japan), and acclimated to the laboratory for one week prior to initiation of the study. The animals were housed five per cage in principle in conventional room. Mice were given CE-2 (Clea Japan, Inc., Tokyo, Japan) and tap water *ad libitum*.

Chemicals

Allyl chloride was purchased from Wako

Pure Chemical Industries, Ltd. (Osaka, Japan). The purity of the chemical was minimum 98.0%. Analytical grade dimethylsulfoxide (E. Merck, Darmstadt, Germany) was used for solvent.

Treatments

All mice were weighed and randomly distributed among six experimental groups (five treatment groups and one control group). Five animals were assigned to each group in principle. In each treatment group, mice were given a single dose of 496 mg/kg, 600 mg/kg, 720 mg/kg, 864 mg/kg and 1037 mg/kg of allyl chloride by subcutaneous injection. In the control group, mice were given the same volume of dimethylsulfoxide only by the same manner.

Each group of mouse was observed periodically and weighed every day. Mice were sacrificed using ether anesthesia seven days after the injection. Heart, lung, liver, spleen, kidneys, testes and epididymes were weighed and fixed in Bouin's solution overnight. They were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopic examination.

Calculation of LD_{50}

LD_{50} was calculated using Litchfield-Wilcoxon's method from number of mice of each experimental group died by the 7th day after the injection of allyl chloride.

Results

LD_{50} of allyl chloride in ICR mouse by subcutaneous injection

Table 1 shows the number of mice of six experimental groups died by the 7th day after the injection of allyl chloride. All deaths during the observation period occurred within 24 hours. LD_{50} was calculated 621 mg/kg (95% confidence interval: 522-739 mg/kg) from this result using Litchfield-Wilcoxon's method.

Behavioral study

In mice of all treatment groups, slight narcosis was noted a few hours after the injection. The narcotic effect disappeared within 24 hours in surviving mice, and there was a dose-effect relationship between the dose of allyl chloride and the duration of the narcotic effect. However, no definite paralytic or ataxic gait was detected. No other behavior abnormality were observed. No significant weight loss was also noted in mice of the treatment groups survived for seven days after the injection.

Table 1 Mortality of mice by the 7th day after the injection of allyl chloride (AC).

Dose of AC (mg/kg)	Mortality
(Control)	0/6
496	1/5
600	3/5
720	3/5
864	4/5
1037	5/5

Macroscopical findings

All mice died within 24 hours after the injection showed a extensive hemorrhage in the lung and some animals showed a mild splenomegaly. No particular macroscopic changes were observed in the other organs examined.

In six of the nine mice of the treatment groups sacrificed on the 7th day after the injection, testicular atrophy was seen, and the degree of this lesion varied with individuals from moderate to severe (Table 2). The difference of testicular weight between these nine mice (0.077 ± 0.029 g, $n=9$) and control mice (0.101 ± 0.013, $n=6$) was marginally significant ($p=0.09$). No particular macroscopic changes were observed in the other organs other than testes.

Light microscopical findings

In all mice died within 24 hours after the injection, there was a marked congestion with severe hemorrhage and edema in the lung. Liver and kidney damages were also found, and these were characterized by the dilated sinusoids, degenerative change of hepatic cells, and focal necrosis in the liver; and necrosis of

Table 2 Incidence of testicular atrophy in mice survived by the 7th day after the injection of allyl chloride (AC).

Dose of AC (mg/kg)	Incidence of testicular atrophy	Testicular weight (g ; mean(SD))
(Control)	0/6	0.101(0.013)
496	2/4	0.077(0.029)*
600	2/2	
720	1/2	
864	1/1	

*All nine mice were included irrelevant to existence of testicular atrophy.

epithelium in convoluted tubules of the kidneys. However, the degree of the lesions in the liver and the kidneys varied with individuals and a dose-effect relationship was not observed between the does of allyl chloride and the degree of these lesions.

In mice of treatment groups sacrificed on the 7th day after the injection, histopathological changes were found only in the testes and these lesions could be classified into two types.

Figure 1 shows seminiferous tubules of the control mouse. All types of germ cells were regularly arranged and no histopathological changes were found.

Figure 2 shows one type of the testicular lesion in a mouse of the treatment group. Seminiferous tubules became atrophic, all types of germ cells lining the tubules have degenerated and exfoliated, and mature spermatids were rarely observed. "Empty" tubules were also found, though Sertoli cells and spermatogonia remained relatively well even in the severely damaged seminiferous tubules. Mild proliferation of Leydig cells occurred and sometimes mitotic figures could be observed in these cells. Besides, appearance of polynuclear giant cells in tubules were frequently observed (Fig. 3).

Figure 4 shows another type of the testicular lesion in a mouse of the treatment group. Seminiferous tubules did not reduced their diameters, though all type of cells, including Sertoli cells, became necrotic almost keeping

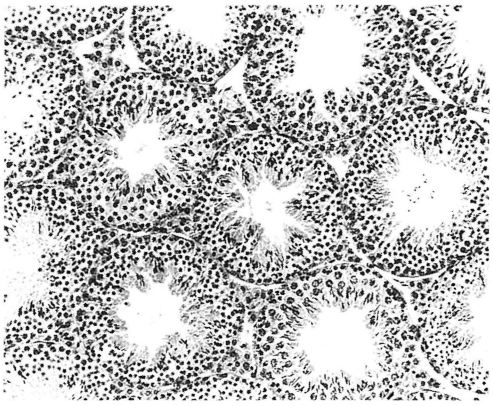

Fig. 1 Testis of the control mouse (134×)

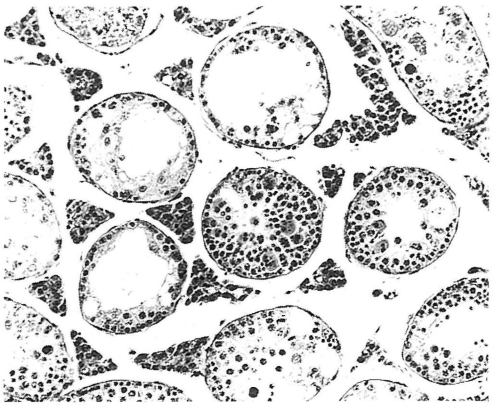

Fig. 2 Testis of a mouse of the treatment group (600mg/kg of allyl chloride). Atrophy of the seminiferous tubules, degeneration and exfoliation of the germ cells were observed (134×)

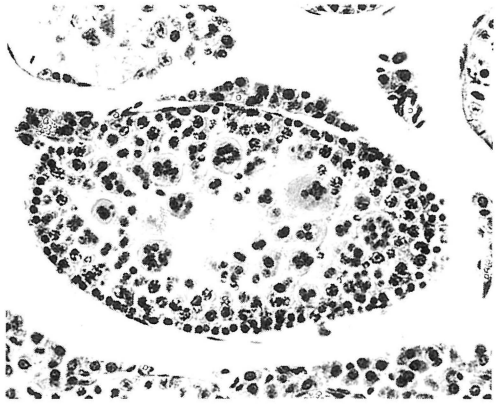

Fig. 3 Polynuclear giant cells in a seminiferous epithelium of a mouse of the treatment group (600 mg/kg of allyl chloride, 250×)

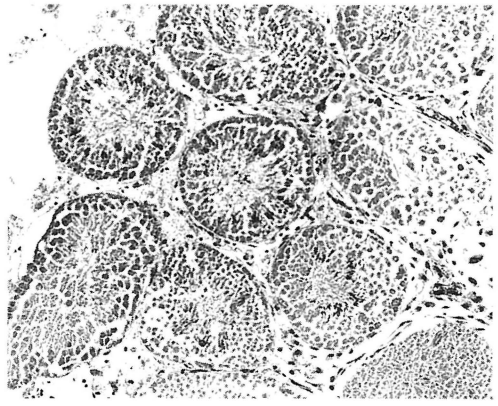

Fig. 4 Testis of a mouse of the treatment group (864 mg/kg of allyl chloride). All type of cells, including Sertoli cells and Leydig cells, became necrotic (134×)

their arrangement in tubules and nuclei of mature spermatids just before spermiation became pyknotic. Leydig cells also became necrotic and only fibroblasts could be observed in the interstitium. This type of the testicular lesion could be found in mice injected with lower dose of allyl chloride, though less frequently. Therefore, this type of testicular lesion seems to be more severe than another lesion mentioned before.

There were three mice which showed no macroscopic testicular atrophy. No histopathological changes were also found in almost all seminiferous tubules in them, though appearance of polynuclear giant cells (Fig. 5) and degeneration of mature spermatids were observed in a few tubules.

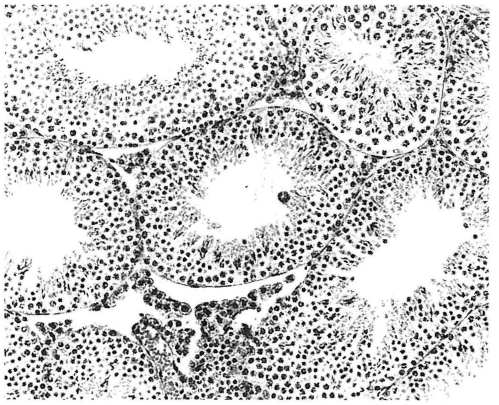

Fig. 5 Polynuclear giant cell in a seminiferous epithelium of a mouse which did not show a macroscopic testicular atrophy (496 mg/kg of allyl chloride, 134×)

Discussion

In the acute toxicity studies which have ever been conducted, the oral LD_{50} of allyl chloride were reported to be 425 mg/kg body weight in mice and LD_{50} for two-hour inhalation exposure were reported to be 11,500 mg/m^3 in female mice. In the present study, we evaluated the acute toxicity of allyl chloride in mice by subcutaneous injection and LD_{50} for subcutaneous injection was calculated 621 mg/kg body weight in mice.

Slight narcotic effect was observed in the behavioral study. Besides, histopathological examination showed a marked congestive change in the lung. There were also a degenerative and a necrotic lesion in the liver and the kidneys to a various degree. Those were already reported by Adams et al.[1] who had examined the acute toxicity of allyl chloride by inhalation exposure. However, congestive change in the lung observed in this study were

probably due to a circulatory collapse rather than due to the direct effect of allyl chloride on the lung, because it was administered by subcutaneous injection in this study. An existence of mild splenomegaly along with pulmonary congestion, and a lack of pulmonary lesions in surviving mice also support this hypothesis.

In the present study, we also found the testicular toxicity of allyl chloride in mice by subcutaneous injection. The testicular lesions could be classified into two types. In one type of the testicular lesion, seminiferous tubules became atrophic, all types of germ cells lining the tubules have degenerated and exfoliated, and mature spermatids were rarely observed, though Sertoli cells and spermatogonia remained relatively well even in the severely damaged seminiferous tubules. Appearance of polynuclear giant cells in tubules and a proliferation of Leydig cells were also observed. Another type of the testicular lesion was characterized by the necrosis of all type of cells, including Sertoli cells, keeping their arrangement in tubules. Leydig cells also became necrotic in this type of the lesion. This is the first study reporting the testicular toxicity of allyl chloride. Ahmad et al.[2] administered a daily dose of 50 mg/kg of allyl chloride to adult Long-Evans male rats by subcutaneous injection for one, three, or six months, though no significant changes were observed on the male reproductive system. Total dose they administered was maximum 18 times more than that of our study. However, single dose they used was 10 times less than ours and probably this is the reason why they could not find the testicular toxicity of this substance. Allyl chloride was an alkylating agent and known to cause DNA damage[4),10]. Besides, it is a low molecular substance (molecular weight: 76. 5) and probably passes through the blood-testis barrier. Therefore, allyl chloride may affect germ cells in the seminiferous tubules directly and cause DNA damage. However, testicular damages observed in this study was so severe that it is difficult to estimate the mechanism of the testicular toxicity of this compound. Now we are conducting the testicular toxicity studies of allyl chloride at the lower dose to elucidate this mechanism.

Acknowledgement

The present study was performed with co-authors Mr. Yasuhiro Itonaga and Mr. Hazime Komatsu in the Laboratory Course in Faculty of Medicine, Kyushu University in 1993. And the authors wish to thank Ms. Yosiko Hirose for her skillful assistance.

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(和文抄録)

塩化アリルのマウスへの皮下投与による急性毒性実験

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雄のICRマウスを五群に分け，それぞれに対して塩化アリルを496 mg/kg，600 mg/kg，720 mg/kg，864 mg/kg，1037 mg/kgずつ皮下に一回投与し，その急性毒性について検討を行った．

投与後7日間に25匹中16匹のマウスが死亡し，これから塩化アリルの皮下投与によるLD_{50}は621 mg/kg（95%信頼区間：522 mg/kg～739 mg/kg）と計算された．これらのマウスの病理組織学検査で，肺には肺水腫および肺胞内への出血を伴った著明なうっ血所見を認め，また肝臓では類洞の拡張，肝細胞の変性・巣状壊死を，また腎臓では尿細管の壊死を認めた．

投与後7日目に剖検したマウスでは病理組織学検査で精巣にのみ病変を認めた．精巣の病変は大きく二型に分けられ，一方の病変では精細管生殖細胞の変性・脱落および生殖細胞由来と考えられる多核巨細胞の出現を認め，間質のLeydig細胞は軽度の増生を示していた．もう一方の病変ではSertoli細胞も含めた精細管内のすべての細胞の壊死が認められ，間質のLeydig細胞も壊死に陥っていた．なお，この論文は塩化アリルの精巣毒性に関する初めての報告である．

本研究は，九州大学医学部の平成5年度学生基礎配置において共同執筆者である糸永康洋君，小松　一君の両学生らとともにまとめたものである．